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Benzylic rearrangement stable isotope labeling for quantitation of guanidino and ureido compounds in thyroid tissues by liquid chromatography-electrospray ionization mass spectrometry

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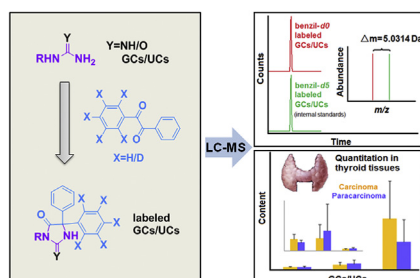
HIGHLIGHTS

- A common reagent, benzil-*d0*/*d5* was employed to label the GCs and UCs through BRSIL.
- The benzil-*d0*/*d5* labeling improved the retention behavior in RPLC and increased the sensitivity by ESI MS detection.
- BRSIL coupled with LC-ESI MS was applied to the qualitation and quantitation of GCs and UCs in thyroid tissues.

GRAPHICAL ABSTRACT



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ABSTRACT

Benzylic rearrangement stable isotope labeling (BRSIL) was explored to quantify the guanidino and ureido compounds (GCs and UCs). This method employed a common reagent, benzil, to label the guanidino and ureido groups through nucleophilic attacking then benzylic migrating. The use of BRSIL was investigated in the analysis of five GCs (creatine, L-arginine, homoarginine, 4-guanidinobutyric acid, and methylguanidine) and two UCs (urea and citrulline). The labeling was found simple and specific. The introduction of bi-phenyl group and the generation of nitrogen heterocyclic ring in the benzil-*d0*/*d5* labeled GCs and UCs improved the retention behaviors in liquid chromatography (LC) and increased the sensitivity of electrospray ionization mass spectrometry (ESI MS) detection. The fragment ion pairs of *m/z*

Abbreviations: BRSIL, benzylic rearrangement stable isotope labeling; GCs and UCs, guanidino and ureido compounds; LC, liquid chromatography; ESI MS, electrospray ionization mass spectrometry; DAMO, diacetyl monoxime; TSC, thiosemicarbazide; MG, methylguanidine; GBA, 4-guanidinobutyric acid; CT, creatine; Arg, L-arginine; Harg, homoarginine; Ala, alanine; Lys, lysine; Asn, asparagine; Cit, citrulline; EIC, extracted ion chromatograms; QTOF, quadrupole time of flight; LOD, limit of detection; SD, standard deviation; NMR, nuclear magnetic resonance.

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Guanidino group
Ureido group
Liquid chromatography-mass spectrometry
Benzylic rearrangement
Thyroid tissue

182/187 and *m/z* 210/215 from the benzil-*d0/d5* tags facilitated the discovery of potential GCs and UCs candidates residing in biological matrices. The use of BRSIL combined with LC-ESI MS was applied for simultaneously quantitation of GCs and UCs in thyroid tissues. It was demonstrated that nine GCs and UCs were detected, six of which were further quantified based on corresponding standards. It was concluded that five GCs and UCs (L-arginine, homoarginine, 4-guanidinobutyric acid, methylguanidine, and citrulline) were statistically significantly different ($p < 0.05$) between the para-carcinoma and carcinoma thyroid tissue samples.

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1. Introduction

Of the large number of small water-soluble compounds, the guanidino compounds (GCs) and ureido compounds (UCs) are widespread in fungi [1], plants [2], animals [2] and humans [3]. They are known to have important biological activities and take participation in many biochemical processes. The most notable two are the urea and guanidine cycles [4]. GCs and UCs involved serve an important role in the metabolism of nitrogen-containing compounds by animals and are the main nitrogen-containing substances in mammals [5]. GCs and UCs have been under study for quite a long time in the fields of toxicology [6,7] and biomedicine [8]. Some of GCs such as guanidine, methylguanidine, guanidinosuccinic acid etc. are classified as uremic toxins because they accumulate in the biological fluids of uremic patients and indicate symptoms of uremia [9–11]. Plasma citrulline succeeds in discriminating, as an independent indicator, transient as opposed to permanent intestinal failure with a markedly high level of sensitivity and specificity [12–14]. Therefore, measuring and mathematically modeling changes in the levels of GCs and UCs found in biological fluids offers deep insight into the effects of diet, drugs, and diseases.

GCs and UCs usually present at low concentrations in the complicated biological fluids. It is necessary to explore a sensitive, specific, and accurate analytical technique capable of their measurements and quantifications. Due to the high polarity and the lack of chromophoric groups of these water-soluble small molecules, the methods for GCs determination are frequently relied on the various derivatizations combined with the different analytical tools like spectrophotometry, liquid chromatography (LC) [15–18], capillary electrophoresis [19], and gas chromatography [20,21]. The derivatizing reagents mainly include thymol-sodium hypobromite [22], pyridoin [23], benzoin [24–26], ninhydrin [15], hexafluoroacetylacetone and ethyl chloroformate [27], glyoxal and ethyl chloroformate [28], and glyoxal [29]. Mass spectrometry (MS), as a powerful and versatile analytical tool, also has been coupled to gas chromatography separation or LC separation (LC-MS) [30,31] for the identification and quantification of GCs. Compared to GCs, reports focusing on the detection of UCs are few. The colorimetric analysis is frequently used, which the color mix, such as diacetyl monoxime (DAMO)-antipyrine [32] and DAMO-thiosemicarbazide (TSC) [33] can react with ureido group. Nevertheless, this method cannot provide the detail of structural information, which leads to the uncertainty in the subsequent analysis. In some cases [34,35], simultaneous detection and quantification of multi-compounds are needed. This is extremely important in the biomedical science research area. Like the metabolic diseases, their diagnoses tend to rely on the levels and changes of multi-endogenous metabolites. Since GCs and UCs participate in the biochemical processes [1,36,37], simultaneous detection of these two kinds of compounds would be of great clinical value. Till now, few relevant analytical methods have been reported.

One of the common bioanalytical techniques for metabolites analysis is LC method coupled to electrospray ionization mass spectrometry (LC-ESI MS) [38,39]. However, the accurate quantification based on LC-ESI MS faces several challenges, including matrix effect, calibration, and the determination of analytical and biological variation [40,41]. An alternative strategy is to use chemical labeling that introduces a stable isotopic tag to metabolites of interest. The use of stable isotope labeling analogues as internal standards facilitates the quantification with improved accuracy and specificity [42,43]. It normalizes the MS intensity of analytes to their isotopic analogues and thus effectively compensates for the matrix effect, ion suppression from other co-eluting analytes, and variations caused by sample preparation, injection, and instrument parameters [44–46]. This strategy has been applied successfully to the quantification of the interest in biological samples, like amine-containing metabolites [47–49], thiol-containing compounds [50,51], amino acid [52,53], fatty acids [54,55], and fatty alcohols [56].

In this paper, we reported a novel strategy for simultaneous quantification of GCs and UCs using benzylic rearrangement stable isotope labeling (BRSIL). The labeling of the guanidino and ureido groups with benzil was explored. It was determined that the benzil-*d0/d5* labeled GCs and UCs could improve the retention behaviors in reversed-phase LC and increase the sensitivity of ESI MS detection. The fragment ion pairs from the benzil-*d0/d5* tags contributed to discover the potential GCs and UCs candidates in biologic matrices. Finally, the applicability of this newly developed method was validated to simultaneously analyze GCs and UCs (free and modified) in thyroid tissues.

2. Materials and methods

2.1. Chemicals and reagents

Methylguanidine (MG) hydrochloride, 4-guanidinobutyric acid (GBA) and formic acid were purchased from Sigma–Aldrich (St. Louis, Missouri, United States). Creatine (CT) was purchased from Alfa Aesar (Massachusetts, United States). L-Arginine (Arg), homoarginine (Harg), alanine (Ala), lysine (Lys), asparagine (Asn), and urea were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Citrulline (Cit) and benzil were purchased from TCI Development Co., Ltd. (Tokyo, Japan). Methanol and dichloromethane were of HPLC grade and were purchased from Merck KGaA (Darmstadt, Germany). Deionized water was produced by a Direct-Q water purification system (Millipore, El Paso, TX, USA). All other chemicals and solvents used were of analytical grade. Stock solutions of GCs and UCs were prepared in deionized water at a concentration of 0.1 mol/L (M). Working solutions were obtained by appropriate mixing and dilution of the stock. Derivatizing reagent solution of benzil was prepared in methanol at a concentration of 20 mM.

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